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Milk and Bact.

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BACTERIA IN MILK.

A SUMMARY

OF THE

Present Knowledge Concerning Their Source and Significance.

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The following summary on "Bacteria in Milk" was prepared by Veranus A. Moore, Professor of Comparative Pathology and Bacteriology at the New York State Veterinary College, Cornell University, Ithaca, N. Y., at the request and under the direction of this Department. This was found necessary in order to meet certain questions arising under section twenty-two of the Agricultural Law.

CHARLES A. WIETING,

Commissioner of Agriculture.

BACTERIA IN MILK.

It was not many years ago that milk, freshly drawn from the udders of healthy cows, was supposed to be free from bacteria. It was known, however, that it was subject to many and often unexpected changes which impaired its value or rendered it altogether unfit for human consumption. The conditions themselves under which the milk was produced and kept did not seem to be sufficient to explain these often observed phenomena. In consequence of this, many theories were advanced to elucidate the exceedingly practical yet troublesome problems found in the occasional rapid souring, the development of bad taints and flavors, the appearance of disagreeable fermentations, and all too often the seemingly disease engendering properties in the milk and its products with which the dairyman, milk dealer and consumer have to contend. With the advent of bacteriology, however, rational explanations for these various evils began to appear. Already investigations concerning the bacterial content of milk have not only afforded an explanation for many of these troubles but they have also pointed out with unerring accuracy certain precautions that must be taken, and procedures that must be observed if they are to be avoided. With a knowledge of the cause of these changes there have come definite methods for their prevention.

It is unfortunate that in its beginning bacteriology became known as the science which dealt almost, if not entirely, with the cause of the infectious diseases of man and of animals. On this account the opinion is quite prevalent that the only function of bacteria is to produce disease, and that in the absence of a diseased condition there are no bacteria. Because of the minute structure of these organisms it is easy for those not trained in this new field in biology to entertain such views. In

consequence of this, the action that is being taken by certain boards of health pointing to the rejection of milk containing an excessive number of bacteria, regardless of their species, and coming from cows in perfect health, is difficult to understand unless the full significance of these micro-organisms is clearly recognized. The truth is, that of the hundreds of species of bacteria freely distributed everywhere in external nature and which may find their way into milk but very few of them are capable of directly producing a diseased condition in man or animals. The others are either harmless or, by virtue of their effects upon the constituents of milk, they produce by-products that may be poisonous or injurious to the consumer. The problems arising from a knowledge of bacteria in milk that have already been studied but which need to be more thoroughly investigated, resolve themselves very naturally into three groups, namely:

1. Those which pertain to the manner in which bacteria gain access to the milk.

2. The effects bacteria produce (a) on the milk itself and (b) the effect of the changed milk upon the consumer.

3. The methods of handling milk in order to keep the number of bacteria and the products of their growth at a possible minimum point.

It has been known for several years that market milk as it is delivered to customers contains large, but variable, numbers of bacteria. The results of quantitative bacterial examinations of milk in various cities, both at home and abroad, show that where reasonable cleanliness in the care of the cows, stables, and milk is observed, and the milk is delivered within a few hours after it is drawn, that the number of bacteria it contains ranges from a few hundred to 100,000 per cubic centimeter. In other instances, more especially in the large cities, where the time between milking and the delivery of the milk is necessarily much longer, there is a larger number of bacteria present. It is reported in one instance from a European city that the milk contained 180,000,000 bacteria per cubic centimeter at the time

it was delivered to the regular customers. In New York and other large cities the milk that is carelessly kept and dispensed from the "corner grocery" contains millions of bacteria per cubic centimeter. Without entering further into particular cases, suffice it to say, that it can be positively stated that market milk always contains a greater or less number of bacteria. When reasonable precautions are taken, and the milk is delivered within a few hours after it is drawn the number is comparatively small, but when the opposite is permitted the number of bacteria is correspondingly increased.

Source of Bacteria in Milk.

When bacteria were first found to exist in mixed milk it was supposed that a few of them had gained entrance from without and that they had rapidly multiplied, giving rise to the large numbers found at the time of the examinations. The inquiries into the ways and means by which these micro-organisms could gain entrance to the milk show that, for the greater part, these external sources consist (1) of particles of dirt, laden with bacteria, which fall into the milk utensils before and after the milking process, (2) the dirt and filth which fall at the time of milking into the milk pail from the skin of the udder and groin of the cow, (3) the dirt otherwise introduced from the hands and clothing of the milker and (4) the bacteria that remain in the utensils from improper cleaning.

The number of bacteria that ordinarily fall into the milk pail from the skin of the udder and groin of the cow during the milking process is much larger than may be supposed. To obtain definite information on this point both Ward and Hunziker have exposed gelatin plates under the udder, between the arms of the milker, and over the milk pail, for different periods of time. They made parallel tests with cows ungroomed and with those which had the hair and skin brushed and moistened just before milking. The number of bacteria which fall into the milk pail, as indicated by the number of colonies which developed on the plate cultures, was estimated to vary from

a few to many thousands. The number falling from the unmoistened skin was exceedingly large.

It was finally shown that milk when drawn with all the known precautions against external contamination still contained a variable number of bacteria. This led to many careful investigations which resulted in showing that there are two distinct sources from which bacteria gain entrance to the milk, namely: (1) from external sources as already stated, and (2) from the milk ducts of the udder itself. It was easy to understand how readily bacteria could gain access to mixed milk through the external channels of contamination, but to establish a general belief that milk as drawn from the healthy udder contained bacteria required that their presence in the milk thus drawn should be demonstrated.

Leopold Schultz was among the first who carefully conducted experiments to determine the extent of the milk duct infection. In 1892 he examined the milk bacteriologically at the first of the milking, in the middle of the milking and at its close. The results of the different tests were quite uniform. The first milk drawn (fore milk) usually contained numerous bacteria, as many as 97,200 per cubic centimeter being found in one instance. The number of bacteria in the milk at the middle of the milking was much less, the average being 9,000 per cubic centimeter. The cultures from the milk at the close of the milking remained clear in some cases, in others from 500 to 600 bacteria per cubic centimeter were found.

Gernhardt made a series of similar examinations, but with quite different results. He found that the number of bacteria in the milk was at first moderately high, often reaching 600,000 per cubic centimeter. This number rapidly increased, so that the examination made at the middle of the milking showed as high as 7,000,000 bacteria per cubic centimeter, but toward the close the number diminished, so that frequently they were absent from the milk taken at the finish. His suggestions concerning the results are, that the bacteria grew up through the milk ducts of the teats into the udder itself, where they multiply rapidly in the acini of the mammary gland, and that they

are not readily removed from their inner recesses. Gernhardt found many irregularities in his figures, which he explained on the supposition that in the cases where the number of bacteria were exceedingly large, masses or clumps of the organisms were washed from the milk ducts into the milk.

Russell states in his Dairy Bacteriology, 1894, that he has been unable to find the last milk drawn from the udder to be free from bacteria.

At the meeting of the Association of American Physicians held in Washington, D. C., in 1894, Dr. T. M. Rotch presented a paper giving the results of the experiments which he, assisted by Dr. Austin Peters, had made near Boston, Mass., to determine the number of bacteria in freshly drawn milk. The fore milk and the milk near the close were taken, and also the milk drawn through a sterile canula passed into the milk duct nearly if not quite through the teat. Four cows were thus examined with the following results:

	A.	B.	C.	D.
1	141	167	19	58
2	0	0	1	2
3	0	6	0	0
4	0	0	1	2

1 represents the milk in the first half of milking and drawn by hand into sterile bottles.

2 represents milk drawn through sterile canula into bottles.

3 and 4 represent milk drawn by hand after more than half the udder had been emptied.

The columns marked A, B, C, D, represent the milk from the four cows, the figures showing the number of colonies of bacteria in the plates made from each specimen of milk.

Rotch concluded from these experiments that practically sterile milk could be obtained by rejecting the first or fore milk from each teat.

In 1895 the writer reported the results of an examination of the fore milk and strippings of nine cows.* Separate examina-

*The method followed was very simple. In collecting the milk the teats and udders were washed with a solution of 1 to 1,000 corrosive sublimate. The hands and arms of the milker were likewise disinfected and care was exercised to prevent the stirring up of dust. The milk was drawn directly into sterilized bottles. From 5 to 10 c. c. of the fore milk was taken in separate bottles from each quarter of the udder and about 50 c. c. of the strippings except in the first five cases. The milk was taken directly to the laboratory and agar plates were made with definite quantities (from 0.1 to 1 c. c.) of it. The plates were incubated at about 36° C.

tions were made of the milk from each quarter of the udder. The results obtained from the agar plate cultures are appended.

THE NUMBER OF BACTERIA PER CUBIC CENTIMETER IN FRESHLY DRAWN MILK TAKEN UNDER ASEPTIC PRECAUTIONS, AS DETERMINED BY THE NUMBER OF COLONIES ON AGAR PLATES.

COW NUMBER.	COLONIES IN FIRST MILK.				COLONIES IN LAST MILK.			
	QUARTER OF THE UDDER.							
	1.	2.	3.	4.	1.	2.	3.	4.
1	0	6	6,625	1,750	......	......	......	......
2	7	Fungi.	0	Fungi.	......	......	......	......
3	180	3,840	1,200	18	......	......	......	......
4	5	1,200	*a*	5	......	......	......	......
5	0	3,840	6	12	......	......	......	......
6	Very many.	Very many.	0	600	1,800	2,400	0	0
7	Very many.	7,200	8,400	940	546	0	504	0
8	455	0	390	0	2	0	0	0
9	4	8	0	8	0	4	0	0
9 (later data)	230	5	120	5	0	0	0	0

a Surface of plates covered with a spreading growth of bacteria.

In all, 20 apparently different species of bacteria were isolated. Of these, 3 were streptococci, 13 micrococci, and 4 bacilli. It is important to state, however, that the great majority of these species appeared in the cultures but once or twice. About three species of micrococci were the predominating forms and they were almost always present. Among the bacilli, *Bacillus cloacae* occurred in one specimen. It is possible, as it appeared but once, that is was an accidental contamination from external sources. This was the only gas producer in the entire series. Nine of the apparent species were aerobic, indicating their inability to multiply high up in the glandular tissue and suggesting their restriction to the lower milk ducts. The other species were facultative anaerobes and could have multiplied much higher up in the teat or milk ducts of the udder.

With one exception the organisms isolated fermented lactose in both bouillon, to which chemically pure lactose had been added, and in milk itself, giving a decidely acid reaction. Six of the twenty species produced a firm coagulation in milk within twenty hours. The others precipitated or coagulated the casein in from four to ten days. A few of the milk cultures

emitted a very strong "sour milk" odor. The odor given off from the others was either not perceptible or of a pungent or disagreeable nature. Eleven of the species liquefied gelatin. In some instances liquefaction occurred rapidly, in others more slowly, several days being required to complete the process.

The pathogenesis of these organisms was tested by the subcutaneous inoculation of 0.5 c. c. of a fresh bouillon culture into guinea pigs. In every instance the animals remained well. In fact, a perceptible local lesion was not produced in any case.

Bolley and Hall, of the North Dakota Experiment Station, made a qualitative test of the fore milk of 10 healthy cows. They isolated 16 species, 12 of which produced an acid reaction in milk, 3 produced an alkaline reaction, and 1 species failed to cause any appreciable change. They state that gas-engendering bacteria were not found.

In 1898 investigations were begun at the New York State Veterinary College to determine the extent to which bacteria actually invade the healthy udder. As healthy milch cows are rarely killed, it was necessary to use those that were slaughtered after reacting to the tuberculin test. In all of the animals whose udders were used the tubercular lesions were restricted to the bronchial and throat glands, the udders themselves being perfectly normal in appearance. In all nineteen udders have been examined by the writer and Dr. A. R. Ward with the uniform result that bacteria were found in greater or less numbers in different parts of the gland substance.*

*The methods followed in making the examinations may be of interest. Just before slaughtering the cows, samples of the fore milk were taken from which cultures were made. The animals were then milked as dry as possible, under the existing conditions. Immediately after the cow was killed the udder was carefully removed and placed on an inclining table with the base of the udder uppermost. The skin was carefully reflected and with a sterile knife an incision was made extending from the upper part of the udder to the cistern, and of such depth as to expose tissues in the vicinity of the vertical axis of the gland. In making cultures from the glandular tissue, care was taken to prevent milk of the ventral region from coming in contact with the freshly exposed surfaces which normally lie above the cistern. After scorching the exposed surface, bits of tissue were removed with sterile scissors, and transferred to culture media by the use of a flamed platinum loop or forceps. For convenience in noting results the gland was divided arbitrarily into three parts as follows: (A) The lower third including the teat and cistern; (B) The middle third which includes the lower half of the gland proper, and (C) The upper third which includes the remaining portion of the gland (see plate 1). Slant agar tubes or gelatin plates were used. In the beginning both were employed.

The results of the examinations in a few individual cases are appended to illustrate the actual findings.

SUMMARY OF THE RESULTS OF THE EXAMINATION OF UDDER NO. 8.

QUARTER OF UDDER.	Region.	Results.
Right fore	A	Twenty colonies.
Right fore	B	Fifty colonies.
Right fore	C	Fifteen colonies.
Right hind	A	Two colonies.
Right hind	A	Three colonies.
Right hind	B	Eight colonies.
Right hind	C	One colony.
Right hind	C	Three colonies.
Left fore	A	Four colonies.
Left fore	B	Negative.
Left fore	C	Negative.
Left hind	A	Negative
Left hind	B	One colony.
Left hind	C	Eighteen colonies.

SUMMARY OF RESULTS OF THE EXAMINATION OF UDDER NO. 11.

QUARTER OF UDDER.	Region.	Results.
Right fore	A	Numerous colonies.
Right fore	B	Six colonies and one mould.
Right fore	C	Very many colonies.
Right hind	A	Very many colonies.
Right hind	B	Numerous colonies.
Right hind	C	Numerous colonies.
Left fore	A	About twenty-five colonies.
Left fore	B	Numerous colonies. Liquefying.
Left fore	C	Very slight growth.
Left hind	A	Numerous colonies.
Left hind	B	Tissue ocher colored, five isolated colonies.
Left hind	C	Numerous colonies.

More recently Hunziker has found variable numbers of bacteria in the fore milk and almost always in the strippings.

During the last year he has made a large number of examinations of milk especially to determine the number of bacteria it contained at the time it was drawn. The samples of milk examined were taken from separate cows. In some cases the milk was drawn under aseptic precautions; in others the cows were milked directly into pails without first cleaning and moistening the skin of the udder. Some of the samples were taken from the mixed milk of a number of cows when milked under the ordinary conditions. A few of the results of these determinations are appended.

TABLE SHOWING THE NUMBER OF BACTERIA PER CUBIC CENTIMETER IN MILK IMMEDIATELY AFTER IT HAS BEEN DRAWN.

SAMPLE.	SAMPLES OF MILK FROM SEPARATE COWS.		Samples from mixed milk drawn without aseptic precautions.
	Drawn aseptically.	Drawn without aseptic precautions.	
1	440	1,212	2,314
2	5,120	85,560	10,060
3	1,715	13,186	2,065
4	2,330	10,340	8,490
5	560	11,500	5,115
6	1,345	......	5,055

Simon has recently published the results of the examinations of the udders of healthy cattle in which he failed to find bacterial invasion. The udders, however, were not functionating at the time the cows were killed, so that the conditions were different from those under which udder examinations have been made in this country. With this exception the results are uniform in showing that milk contains a greater or less number of bacteria at the time it is drawn from the udder. The former belief that the normal udder is sterile and that freshly drawn milk is free from bacteria is no longer tenable.

A further and exceedingly practical illustration of the influence of the udder invasion on the bacterial contents of mixed milk is found in the experience of certain companies that are putting on the market as clean and pure a milk as it is possible for them to obtain. Notwithstanding, with their extreme care to prevent contamination the milk continues to contain variable numbers of bacteria. The application of all the known improvements to secure bacteria-free milk, have brought out with startling clearness the fact that it is yet impossible to do so. The results of the daily examinations which the Walker Gorden company had made in the Pepper Laboratory of the University of Pennsylvania of the milk from their Philadelphia dairy are interesting in this connection.

The following is one of their published weekly reports taken at random:*

Saturday, June 3, number of bacteria per cubic centimeter	525
Sunday, June 4, number of bacteria per cubic centimeter	10,050
Monday, June 5, number of bacteria per cubic centimeter	775
Tuesday, June 6, number of bacteria per cubic centimeter	1,150
Wednesday, June 7, number of bacteria per cubic centimeter	2,575
Thursday, June 8, number of bacteria per cubic centimeter	1,200
Friday, June 9, number of bacteria per cubic centimeter	2,000

A matter worthy of consideration in this connection is the apparent fact that certain species of bacteria become localized in the udder. I have already referred to the twenty species of bacteria isolated from the fore milk of nine cows. When, however, the fore milk is rejected and only those organisms are taken into account which invade the milk ducts of the mammary gland itself the number of species in a single gland is found to be small. Bolley and Hall found in a number of examinations but one species common to them all. Ward found that of four or five species existing in the udder of a single cow that but one of them was common to all the four quarters. He has also called attention to the persistence of certain species in the udder. Thus he found, in the udder of one cow, a streptococus which remained without interruption as determined by frequent examinations, for over two years. Frequently this organism seemed to be the only species present. Without entering into further details concerning the subject, let it suffice to say that there seems to be a limited number of species, mostly micro-

* Philadelphia Medical Journal: June 17 (1899) p. 22. adv.

cocci, which invade and remain in the udder. In addition to this, there are many species that may, under favorable conditions, become colonized in the mammary gland and remain there for variable periods of time. Russell had previously pointed out the fact that the species of bacteria in milk vary with the environment and care of the cows.

Moore and Ward found colonized in the udder of a number of cows in a single dairy a bacillus that produced a disagreeable taint and gassy curd when the milk was used in making cheese. These bacilli persisted for several months, although every possible care was exercised in grooming and disinfecting the skin of the udders, and in cleaning and frequent disinfecting the stables. From the results thus far recorded the bacteria found in the udder itself fall naturally in two classes namely: (1) a few species, mostly micrococci, which persist when once they gain entrance and which seem to possess little or no economic importance save that of lactic acid formation; and (2) a large number of species existing in external nature, which may, under suitable conditions become temporarily colonized in the ducts of the mammary gland. The latter species seem to be able to live but a comparatively short time in the milk ducts. Experiments made by Ward to colonize the udder with strange bacteria gave only temporary positive results.

The Significance of Bacteria in Milk.

Numerous and extended investigations have shown that nearly if not all of the changes that occur in milk subsequent to its escape from the udder are primarily caused by bacteria. The most common of these is the simple souring or lactic acid fermentation. In addition to this well known process there are a number of other and more troublesome changes such as the appearance of blue milk, bitter milk, slimy milk, tainted milk, butyric acid fermentation, saponification and still others which are less conspicuous and more rarely found in this fluid. On the other hand, it is known that the proper ripening of

cream with desirable flavors is the result of the activities of certain species of bacteria. In order, therefore, to procure uniform and desirable flavors in butter, it is the practice in some countries to heat the milk sufficiently to kill the existing bacteria and then to inoculate it with a species that will impart to it the desired flavor and odors. Much experimental work along this line has been done in this country by Prof. Conn.

The *lactic fermentations*, or common "souring," of milk is brought about by a number of species of bacteria. Formerly it was supposed that a single species, *Bacillus acidi lactici*, produced this change which consists in the splitting of the milk sugar molecule into carbonic dioxide and lactic acid. It is known, however, that in this process of splitting up the milk sugar other by-products are produced. In the simple lactic type of fermentation these secondary products are not very important. It should be noted, however, that in the souring of milk by different species of bacteria correspondingly different by-products may be produced. In consequence of this, the souring is often accompanied with by-products that are undesirable if not injurious to the consumer. In these cases the deleterious substances are often produced before the quantity of acid is sufficient to cause curdling. In fact these by-products may become harmful while the milk is still considered sweet and wholesome. It is also interesting that the species of bacteria producing lactic fermentation which have been studied by Adametz, Fokker, Leichmann and others in Europe do not seem to be entirely identical with those isolated and studied by Conn and other investigators in this country. The most telling truths which come to us from all these inquiries is that different bacteria causing souring in milk produce very different effects upon the milk itself as shown in the rapidity of the souring and in the types of fermentation accompanying it. Concerning the mixed fermentations there is need for further and more extended researches. Butyric acid formation which plays an important part in causing "rancid" butter is also the product of the activities of a number of species. It is now

supposed to be the end product of a long series of fermentive changes. Bad flavors, taints and odors in milk and its products are among the final results of the growth of certain species of bacteria.

Milk sometimes becomes very bitter owing to the products of certain bacteria multiplying in it. Weigmann, Conn and Freudenreich have isolated and studied a number of such species. A " blue " color is a peculiar condition of milk caused, as Gessard found, by a single species of bacteria, although it possesses a number of varieties. Slimy or ropy milk is another of the many undesirable conditions brought about by micro-organisms. The cause of this particular trouble seems to be due to one species, *Bacillus lactis viscosus*, first isolated and described by Adametz. Several other species have been suggested as the possible cause in certain instances. Loeffler, Guillebeau, Marshall and Ward have written upon this subject. The specific factor (*B. lactis viscosus*) of this trouble exists according to the investigations of Adametz and Ward in water from which it accidentally, or through carelessness in cleansing the milk pails and cans, gains entrance to the milk. To eliminate this trouble it seems to be necessary to simply scald the pails, strainers and cans used in retaining and handling the milk.

Much has been written concerning disease producing bacteria in milk. These belong to two distinct classes namely: (1) the specific bacteria of certain diseases of cattle, which may, if the animal is suffering from disease, gain entrance to the milk. In this class may be mentioned tuberculosis, the " foot and mouth " disease in Europe and possibly anthrax. (2) The bacteria of certain human diseases such as typhoid fever and diphtheria and the virus of scarlatina and measles. A very large number of epidemics of these diseases have been traced to the milk supply through which the infections occurred. The explanation of this is that in cases where these diseases existed among the attendants or in their homes sufficient care was not taken in handling the milk to prevent the entrance of the virus of these

diseases. In case of typhoid fever, the water used in rinsing the utensils may be contaminated. In cases of diphtheria it often happens that those who have recently apparently recovered from the disease, but, who still have the diphtheria bacilli in their throats, are engaged in milking or in otherwise handling the milk, where by sneezing or coughing these bacilli may escape from the throat into the milk. It is the discovery of these possible yet exceedingly easy means of infecting milk, and the finding of cases where there seems to be no doubt that such infection has actually taken place and where the milk has become the channel of infection, that have caused thoughtful people in villages and cities to justly question the safety in using milk that contains a large amount of dirt or an excessive number of bacteria. The objections to it do not rest in the single fact that the dirt and the usual bacteria are necessarily injurious, but that whenever these exist in excessive quantities it is a sure indication of gross carelessness in handling the milk. If people are careless in these matters they are sure to be equally so in case of the presence among them of infectious diseases. The sad experiences of the past are teaching the importance of taking reasonable precautions against the prevention of such maladies.

In cases where bowel trouble follows the use of milk containing many bacteria the immediate cause is quite as likely to be the effect of the acids and other by-products which have been produced in the milk by various forms of fermentation, as by the activities within the digestive tract, of any one or more species of the micro-organisms consumed. We must look to the effect upon the milk itself for the cause of many but not all of these troubles. An analagous illustration is found in the ptomaine poisoning following, in rare cases, the eating of slightly tainted meat or fish. The poisoning comes from the effect of the by-products or ptomaines produced by certain bacteria in decomposing the flesh, and not by a direct result upon the individual of the bacteria themselves.

The actual relation existing between the number of bacteria and the percentage of lactic acid in milk is of interest in this connection. Concerning this, the appended table by Hunziker is especially telling. The data given in the table are emphasized and perhaps more clearly shown in chart V. In making the tests very simple methods were followed. Portions of the same sample of milk were kept at different temperatures and the number of bacteria and the per cent of lactic acid in the milk were determined every three hours. It is of interest to note the variation in the per cent of the lactic acid at different times, especially in the case of the milk kept at the lower temperatures. At the higher temperatures the acid fermentation caused the milk to sour very rapidly.

TABLE SHOWING THE NUMBER OF BACTERIA IN 1 C. C. OF MILK AND THE PERCENT OF LACTIC ACID AT TEMPERATURES RANGING FROM 50° TO 98° F.

HOURS.	KEPT AT 98° F.		KEPT AT 80° F.		KEPT AT 70° F.		KEPT AT 60° F.		KEPT AT 50° F.	
	Number of bacteria.	Percent of lactic acid.	Number of bacteria.	Percent of lactic acid.	Number of bacteria.	Percent of lactic acid.	Number of bacteria.	Percent of lactic acid.	Number of bacteria.	Percent of lactic acid.
Cow warm....	3,746	.0424	3,746	.0424	3,746	.0424	3,746	.0424	3,746	.0424
After 3 hours..	3,214	......	2,460	....	2,660	....	3,034	....	3,180	....
After 6 hours..	73,600	.07208	3,326	.06572	2,220	.06784	3,194	.05936	3,100	.04664
After 9 hours..	Innumerable.	.07208	9,526	.05724	2,674	.06784	3,154	.04876	2,680	.03816
After 12 hours.	....	.10176	33,920	.08480	2,906	.0848	14,420	.08056	3,614	.07208
After 15 hours	....	.0848	270,000	.08480	15,380	.06784	13,370	.07844	4,040	.06996
After 27 hours.	Coagulated.	.5363	Innumerable.	.23744	560,000	.06572	39,720	.06360	4,634	.06996
After 39 hours.	....	....	Coagulated at 30th hour.		Innumerable.	.12296	160,000	.08904	3,100	.07208
After 48 hours.	....	....	....	....	Coagulated.	....	Innumerable.	.46216	3,460	.05936
After 72 hours.	....	....	....	....	....	....	Coagulated.	....	12,740	.06996

Finally we have to consider the safety of the milk from two points of view, namely: (1) the absence of specific disease producing bacteria and (2) the absence of by-products which are produced by many bacteria when the milk is kept under favorable conditions and for sufficient time for their development. It is the exception when the bacteria, which from the very nature of things, are necessarily present in the milk from healthy cows, will produce any harm to the consumer. The number of bacteria in the milk indicate, however, the possible

extent to which fermentation changes have taken place, or, if examined very soon after milking, it is an index to the care which has been exercised to prevent external contamination.

It is well to notice that at first, while the germicidal action lasts, there is no appreciable increase in the acidity. It is also to be seen that the development of acid does not parallel the increase in the total number of bacteria, but during the last 15 to 20 hours before the milk curdles, when the bacteria are already innumerable, the acidity begins to increase continuously. The fluctuations showing in some cases a decrease in acid apparently marring the uniformity of the results, may be explained by the fact that milk contains species of micro-organisms that produce alkaline fermentations. Until the struggle for supremacy of the different species in the milk is ended, it is not improbable that the alkali-producing organisms have an opportunity to grow and in a measure compete with those of other fermentations. As a result their products will necessarily neutralize an equivalent amount of acid, thus reducing somewhat the degree of acidity of the milk. It has been observed that the fluctuations are more frequent and, perhaps, more abrupt in milk drawn under ordinary conditions and kept in cans than milk drawn aseptically and kept in sterile flasks. The former usually contained a much larger number of species of bacteria. Considering then that the milk may contain, and when taken under ordinary conditions almost invariably does contain, some alkali-producing* species, it is evident that their presence and behavior largely govern the extent of the fluctuations in the amount of acidity in the milk.

The Preservation of Milk; the Value of its Germicidal Action and the Effect of Different Temperatures Upon the Multiplication of the Bacteria it Contains.

In adopting methods for keeping milk sweet and suitable for human consumption, dairymen and milk dealers have to meet the single problem of checking the multiplication of the bac-

*Conn. Classification of Dairy Bacteria,

teria it already contains. It has been pointed out that milk as drawn directly from the udder contains a certain number of bacteria. It has also been shown that a large number of bacteria gain access to the milk from without, although this number can be greatly reduced if proper precautions are taken. The evidence thus far collected points to the bacteria which gain access from without as the most likely to cause the unusual and troublesome fermentations. It is also among these organisms that are most often found those species capable of producing disease in the consumer. With a knowledge of these facts, dealers in milk are in a position to put into effective application the best methods for preventing the undesirable fermentations and the accumulation of by-products resulting from them.

The most effective methods of keeping milk is presumably to pasteurize* it at once and keep it in sterile retainers in a cool place. While this method can be applied in certain instances, it is not applicable or suited to the conditions of all milk producers. It is highly recommended for the home treatment of the milk received. When, however, heating is not applied until after many (18-24) hours have elapsed, it is often too late to prevent the development of undesirable by-products owing to the extent of various fermentations which may have already taken place.

The commonly accepted idea is, that as soon as milk is drawn the bacteria already present begin to multiply with enormous rapidity, and that their increase can be checked only by cooling

* Pasteurization may or may not be sterilization. The term has reference to the method used by Pasteur in 1866 for preserving wine. He found that when wine was heated to a certain temperature, about 165° F., it could be kept without the deleterious after fermentation. About ten years later this method was used for preserving milk. When it was found that milk frequently contained disease-producing bacteria, this method was used to destroy them. The clinical experience in using pasteurized milk taught that a temperature of 165° to 180° F. rendered it less easily digested. Then followed a long series of experiments to determine at what temperature, and how long a time, it is neccessary to heat the milk to destrcy the pathogenic and fermenting bacteria. From these experiments it was learned that a temperature of 150° to 155° F. for one half hour was as effective as a short exposure at 165° to 170° F. There are writers, however, who claim that 140° F. is sufficient. Pasteurization has come to mean, therefore, the destruction of *disease-producing*, if present, and the *fermenting* bacteria by means of a low temperature applied for a certain length of time. If only the ordinary fermenting and pathogenic micro-organisms are present, the milk thus treated may be sterilized. If the spore-bearing bacteria, or those possessed of high thermal death point, should be present, this process would not destroy them. After pasteurizing milk it should be kept if possible in a cool place.

the milk down to a low temperature and keeping it there, or by the addition of antiseptics. Contrary to this generally accepted theory Fokker in 1890, Freudenrich and Freeman at later dates, observed that freshly drawn milk possesses a germicidal action. Unfortunately, no definite investigations were made to determine the extent of this action, thus leaving the interesting observations devoid of economic value. Heim, Caro, Weigmann and Hess have published on this subject, more especially concerning the destructive action of milk upon the bacteria of Asiatic cholera. Schottelius studied this action of milk upon diphtheria bacilli. During the last year Hunziker has been engaged in investigations along this line. Neither of these investigators, however, succeeded in finding the definite cause of the germicidal action, but it is presumed that it exists in the serum. Heim attributed this action on the cholera spirilla to be due to the lactic acid. We simply know, however, from the results obtained, that there is in the freshly drawn milk of some cows a substance or a condition that has the power of destroying, or at least preventing the growth of a certain percentage of the bacteria present.

Hunziker has made a careful study of the germicidal action of milk (1) from individual cows, (2) in the mixed milk of the entire dairy, and (3) the influence of different temperatures upon this action. The results of these tests are worthy of a careful study. A sample of the milk from each cow was divided into three parts, placed in sterile flasks, one of which was kept at each of the temperatures of 40°, 55° and 70° F., respectively. Agar plate cultures were made immediately after milking, and at intervals of three hours until 48 hours had elapsed. Fifteen cows were thus tested. The first two, May and Ida, were milked in the ordinary way into separate pails, no special precautions being taken to prevent contamination from without. The samples of the milk from the other cows were obtained under rigid precautions against particles of dust or other contaminations. The teats, the udder and the parts immediately

surrounding the latter, were carefully washed with a 1-1000 solution of corrosive sublimate and then dried with a clean towel. From the moistened udders the milk was drawn into sterile flasks, which were held in a nearly horizontal position to prevent micro-organisms from falling into them. About the same quantity of milk was taken from each teat. A tabulated summary of this experiment is appended.

I. NUMBER OF BACTERIA IN 1 C. C. OF MILK KEPT AT 40° F.

NAME OF COW.	Cow warm.	After 3 hours.	After 6 hours.	After 9 hours.	After 12 hours.	After 15 hours.	After 24 hours.	After 32 hours.	After 48 hours.
May	1,212	1,080	1,220	1,040	1,020	1,120	1,360	1,040	400
Ida	35,560	35,600	27,040	33,900	27,040	32,960	28,820	28,540	26,320
May	440	400	340	340	320	220	120	100	240
Ida	5,120	4,400	4,260	3,620	3,700	3,900	4,000	3,900	3,840
Hilda	1,715	2,100	2,140	2,380	2,510	2,060	2,090	1,720	1,920
Peach	2,330	2,500	2,270	2,400	2,320	2,300	1,880	1,880	1,560
Clover	37,199	28,930	30,060	30,400	27,940	25,940	27,390	27,850	29,770
Rose	500	540	370	600	510	470	340	321	310
Julia	1,345	1,170	1,070	1,120	870	1,120	990	1,060	1,080
Pansy	3,850	3,630	2,870	3,070	2,880	2,750	2,000	2,560	2.170
Rita	1,086	1,000	780	920	1,060	860	940	1,000	1,000
Vina	3,150	2,430	2,270	2,660	2,380	2,490	2,130	1,880	2,040
Chloe	1,706	1,390	1,560	1,550	1,600	1,640	1,730	1,630	1,260
Stella	1,195	960	960	1,220	1,060	1,000	1,320	970	1,230
Dena	4,980	3,690	3,840	3,870	2,670	3,810	2,750	1,290	720
Average	6,759	5,988	5,417	5,929	5,188	5,531	5,174	5,067	4,917

II. NUMBER OF BACTERIA IN 1 C. C. OF MILK KEPT AT 55° F.

NAME OF COW.	Cow warm.	After 3 hours.	After 6 hours.	After 9 hours.	After 12 hours.	After 15 hours.	After 24 hours.	After 32 hours.	After 48 hours.
May	1,212	1,260	1,400	1,500	1,460	1,360	1,080	3,500	17,740
Ida	35,560	25,440	25,740	26,040	26,780	24,460	23,420	22,040	20,040
May	440	320	300	140	220	240	340	340	240
Ida	5,120	3,900	3,460	2,980	2,800	2,920	3,260	3,220	3,240
Hilda	1,715	2,390	2,190	2,180	2,550	2,460	2,290	2,560	5,540
Peach	2,330	2,510	2,540	1,940	2,150	1,970	2,170	2,740	21,800
Clover	37,199	28,340	26,070	29,640	28,670	27,040	24,980	27,320	28,110
Rose	500	600	360	460	400	410	460	500	1,740
Julia	1,345	1,080	990	980	1,400	1,080	1,080	3,110	68,800
Pansy	3,850	3,130	3,280	3,240	3,410	2,970	3,140	3,050	7,200
Rita	1,086	860	1,090	970	1,000	980	780	1,120	1,630
Vina	3,150	2,520	2,550	2,960	2,730	2,810	2,780	2,830	84,000
Chloe	1,706	1,540	2,170	1,780	1,650	1,720	2,000	2,460	2,520
Stella	1,195	1,000	1,150	1,320	1,210	1,180	1,440	1,530	5,490
Dena	4,980	3,730	3,860	3,540	4,120	4,230	3,910	4,670	11,860
Average	6,759	5,241	5,143	5,311	5,370	5,052	4,872	5,399	18,663

III. NUMBER OF BACTERIA IN 1 C. C. OF MILK KEPT AT 70° F.

NAME OF COW.	Cow warm.	After 3 hours.	After 6 hours.	After 9 hours.	After 12 hours.	After 15 hours.	After 24 hours.	After 32 hours.
May	1,212	1,000	1,340	1,860	3,460	3,460	64,000	800,000
Ida	35,560	18,260	12,100	9,020	7,820	6,720	80,000	416,000
May	440	440	280	280	440	11,540	303,600	....
Ida	5,210	3,560	2,120	1,880	1,800	1,240	4,960	58,400
Hilda	1,715	2,330	2,110	10,200	26,860	192,000	1,527,000	....
Peach	2,330	2,230	2,110	2,580	8,870	26,940	420,000	....
Clover	37,199	20,200	15,390	13,820	12,580	16,050	51,200	204,000
Rose	500	400	420	880	980	1,950	13,250	....
Julia	1,345	1,000	1,000	1,200	5,600	17,720	1,600,000	....
Pansy	3,850	3,290	5,470	6,340	5,000	10,620	1,600,000	....
Rita	1,086	1,170	1,230	1,320	1,200	1,120	29,680	26,400
Vina	3,150	2,240	2,520	2,890	3,040	33,140	2,144,000	....
Chloe	1,706	1,440	1,410	1,930	10,840	9,770	192,000	2,800,000
Stella	1,195	1,040	1,060	1,300	3,460	7,840	640,000	....
Dena	4,980	4,440	2,600	3,750	3,660	5,740	180,000	[illegible]
Average	6,759	4,200	3,411	3,947	6,341	23,495	589,980	....

A study of these tables reveals several interesting facts, namely: (1) That there is a great difference in the number of bacteria in the freshly drawn milk of different cows; (2) that there is no increase in the number of bacteria in the milk for at least six hours after it is drawn when kept at a temperature of either 40°, 55° or 70° F.; (3) there is an actual, but usually not large, decrease in the number of bacteria in the milk for the first six hours after it is drawn; (4) the decrease in the number of bacteria, i. e. the germicidal action, is greatest in the milk kept at 70° F.; (5) the decrease in the number of bacteria is much greater in the milk of certain cows than in that from others; (6) the germicidal action is in the beginning more effective in the milk kept at 70° F.; (7) the germicidal action of the mixed milk is not sufficient to render it of practical value in preserving milk for more than from six to nine hours. It is clear that milk cooled and kept at 40° to 55° F. remains unchanged for a much longer time than when a temperature of 70° F. is maintained. At these low temperatures the number of bacteria continues to decrease for a longer time. It is important to note that frequently the number of bacteria begins to increase in the milk kept at 55° F. after about 48 hours, but when the temperature is reduced to 40° F. they do not. A study of charts I to IV inclusive will emphasize these points.

Although for the first few hours the germicidal power is more active in milk kept at 70° F., it has not been determined, but

probably it is true, that in the milk kept at the lower temperatures it continues to act, but in a less degree, for a much longer time. For this reason it seems best, until more definite information on this point is recorded, to cool milk to at least 55° F. as quickly as possible after it is drawn, and *to keep it at that temperature* until it is delivered. If the milk is not to be consumed within 48 hours it is safer to keep it at a lower (40° F.) temperature.

The difficulties involved in procuring milk reasonably free from bacteria in our large cities do not rest entirely with the [illegible]r. The method of transportation is quite as important. Milk may be produced under the best of conditions, cooled at once to the required temperature at which it is delivered for transportation. If, however, its temperature is allowed to rise to 60° F. or above during the time it is in transit, the bacteria present will multiply, producing acids and by-products, so that when delivered the milk has become markedly, if not seriously, changed. As care of the milk required in the course of transportation is simply to have it put in *clean* cans and kept at a low temperature, there is no reason why milk cannot be shipped hundreds of miles and be delivered in a perfectly sweet and wholesome condition. The details of the methods for keeping it at a low temperature must, of course, be worked out by all shippers, as they have successfully been by some milk companies. The only requisite that the consumer demands in the transportion is that the low temperature be maintained.

Summary.

A study of the results of the various investigations included in the preceding pages concerning the bacterial content of milk, together with the requisites in handling it in order that it may be delivered to the consumer in as unchanged a condition as possible, seems to warrant the following deductions:

1. Milk as it is drawn from the udder contains a variable, but rarely an excessive, number of bacteria.

2. Ordinarily the dirt and finer particles of dust that fall into the pail during the process of milking carry a large number of bacteria with them into the milk.

3. If the stables are kept clean, the cows groomed, and the skin of the udder and surrounding parts are carefully moistened before milking, the number of bacteria that gain entrance to the milk from without can be very greatly reduced.

4. The bacteria in the freshly drawn milk do not begin to multiply to any great extent for from 6 to 9 hours when kept at a temperature of 70° F., or below. After that time they multiply very rapidly if the temperature is favorable.

5. If the milk is carefully protected and promptly cooled down to and kept at a temperature of 40° F., the number of bacteria in it will not subsequently exceed the number present at the close of the milking process. This number should not exceed a maximum of 100,000 per cubic centimeter. If intelligent care is exercised, a minimum of at least from 10,000 to 50,000 per cubic centimeter should be maintained. If cooled to 55° F., like results are obtained for at least 36 to 48 hours, and often for a much longer period.

6. An excessive number of bacteria in milk at any time within 72 hours after it is drawn indicates that it has not been properly handled. When this is the case, the milk is liable to contain acids and by-products, which may be injurious to the consumer. There is also the further danger, as exemplified in many epidemics of typhoid fever, diphtheria and other infectious diseases, that the general carelessness in protecting milk against contaminations would permit the entrance into the milk of disease producing bacteria should these be present on the premises where the milk is produced.

7. There is a pronounced germicidal action in the milk of certain cows. It is absent in that from others. In the mixed milk from a dairy it has but a slight value in reducing the actual number of bacteria, but it seems to be the effective agent in preventing the increase of bacteria immediately after the milk is drawn unless it is kept at the body (98° F.) temperature. (See chart V.)

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There is a very voluminous literature on the bacteriology of milk. Hundreds of articles have been published concerning this subject; of these there are a large number containing the results of more or less extended investigations. In the references here appended there are included only a few treating of different phases of the subject and these are largely restricted to American publications. The European literature is especially rich in articles on this subject.

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DESCRIPTION OF CHARTS AND PLATES.

Charts I to IV, inclusive, show in a graphic form the effect of the different temperatures on the increase in the number of bacteria in milk and also the germicidal action of the milk. The cows were all in the same herd, were fed the same, and kept under like conditions. The figures 3 to 48 on the horizontal line at the top of the chart represent the number of hours, the figures 1,000 to 25,000 on the vertical line represent the number of bacteria in 1 c. c. of milk. It will be noticed that the bacteria in the milk kept at 55° F. do not begin to increase in numbers for about 32 hours. When it is kept at 40° F. the number of bacteria either remain the same as at the beginning or they are slightly diminished in numbers. At 70° F. the bacteria multiply rapidly after from 9 to 12 hours.

Chart I. This shows an absence of a germicidal action in the milk of one cow, beyond the point of preventing an increase in the number of bacteria for nearly 9 hours.

Chart II. This shows a moderate germicidal action in the milk of one cow. In this case the germicidal action continued for 15 hours. It was strongest in the milk kept at 70° F. The usual effect was produced by the lower temperatures.

Chart III. This shows a very strong germicidal action in the milk of one cow. The effect was greatest in the milk kept at 70° F., although it was very pronounced in the milk kept at the lower temperatures.

Chart IV. This shows the germicidal action of the mixed milk from an entire dairy. The effect of the different temperatures upon the bacterial growth is also indicated. After 6 hours the diminution of bacteria ceased in milk kept at 70° F. and after 12 hours their multiplication was very rapid. After 32 hours at 55° F. they began to multiply, but at 40° F. they continued to remain stationary.

Chart V. This is a diagram showing the per cent of lactic acid and a number of bacteria in portions of the same sample of milk kept at different temperatures. The figures 3 to 72 on a horizontal line at the top represent the number of hours, the figures 2,000 to 10,000 on the vertical line represent the number of bacteria and the figures .04 to .20 on the inner vertical line represent the per cent of lactic acid. (After Hunziker.)

Plate 1, figure 1. A drawing of a section through the teat and one-quarter of the udder of the cow. It shows the duct in the teat and the larger ones leading from the cistern to the glandular structure of the organ. The parts represented by the letters A, B, C, indicate the arbitrary divisions into which the gland was divided for purpose of examination. (See page 9.) *a*, is the cistern. *b*, larger milk ducts leading into the cistern and extending up into the gland. *c*, secreting portion of

the udder. Figure 2. A drawing of a small area of the secreting portion of the gland magnified.

Plate 2. Figure 1. A diagram representing the composition of milk (after Babcock). Figure 2. A drawing showing the appearance of milk when magnified. The round disk like bodies of different sizes are the fat globules. Figure 3 shows the appearance of cream magnified.

Plate 3. A drawing of bacteria in milk and various forms of bacteria.

Figure 1. A drawing of specimens of market milk containing many bacteria.

Figure 2. A group showing several forms of bacteria. a, b, c and d represent the various forms of micrococci. e, the form most frequently found in the milk and glandular tissue. e, Bacillus coli communis. f, a bacillus with a capsule. g, the bacillus of tetanus (lockjaw). h, represents several forms of spirilla. i, Pseudomonas. j, a spore bearing bacillus.

Figure 3. A group of bacteria of specific infectious diseases which are liable to be carried in milk. a, tubercle bacteria in milk (Crookshank). b, three forms of the bacillus of diphtheria. c, the bacillus of hog cholera, when stained showing the flagella or organs of locomotion. d, the bacillus of typhoid fever.

Figure 4. The bacteria of anthrax with and without spores.

Figure 5. The streptococcus of infectious mastitis in cows. The drawing is made from a cover glass preparation of the thick and blood stained secretion of the gland affected. It represents the chains of streptococci with pus cells and fat globules.

Figure 6. Bacillus lactis viscosus. a, from milk showing the wide capsule. b, showing a polar stain. c, uniformly deeply stained. (After Ward.)

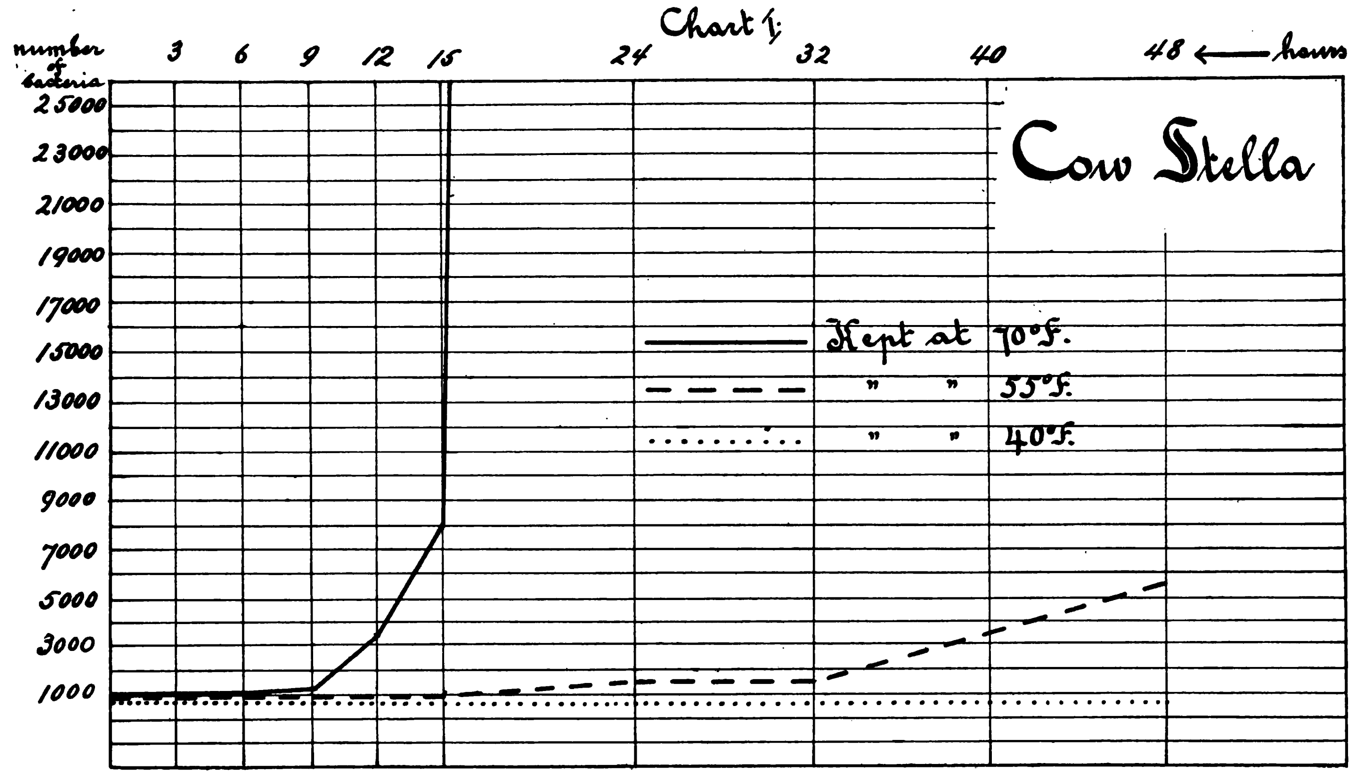


Chart showing the absence of germicidal action in milk of one cow.

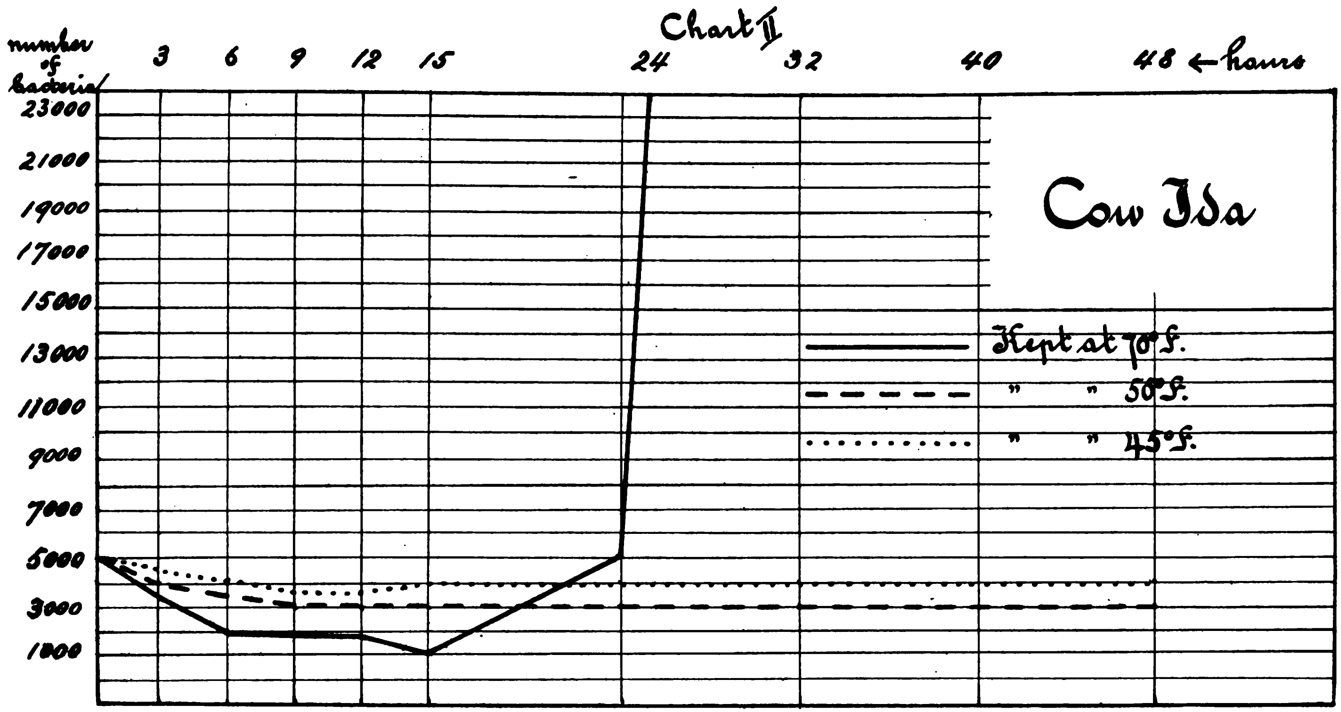


Chart showing germicidal action in milk of one cow.

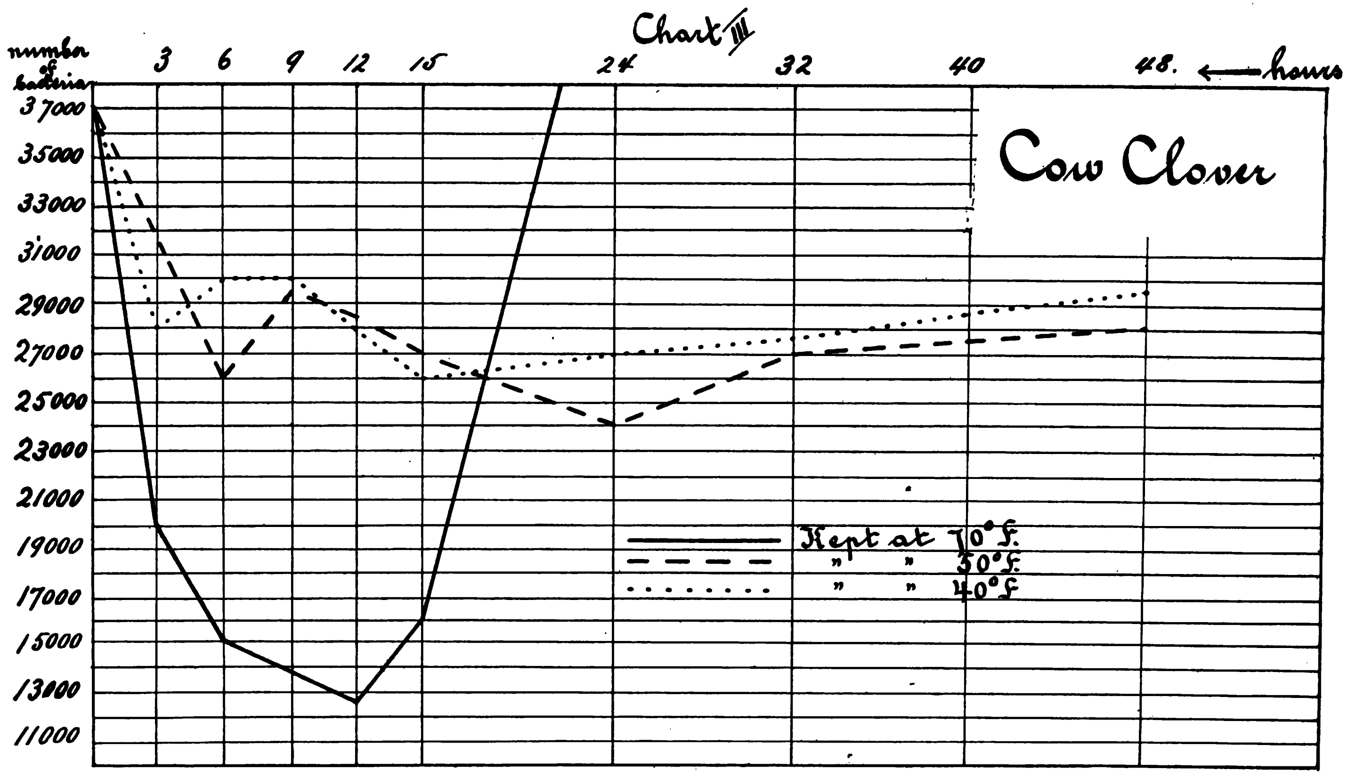


Chart showing the germicidal action in milk of one cow, when kept at low temperatures.

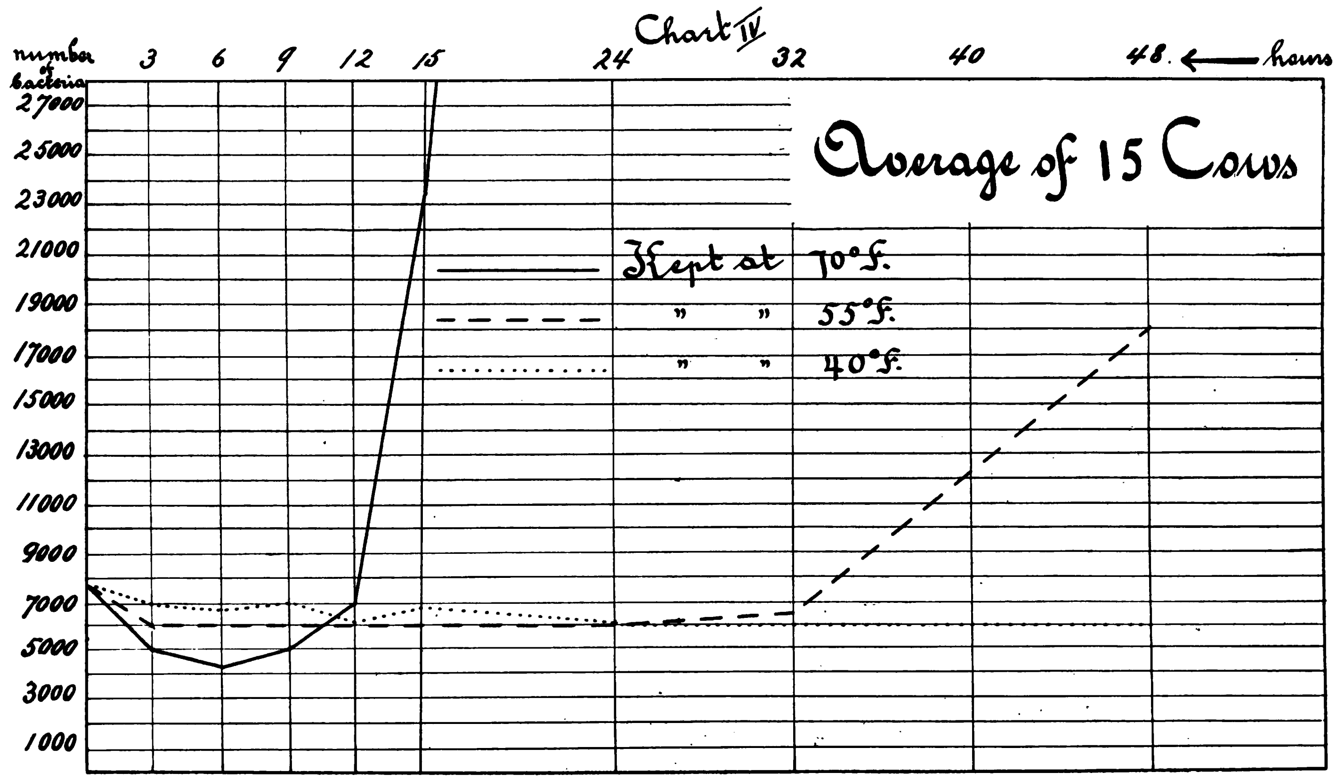


Chart showing the germicidal action of the mixed milk.

Plate II

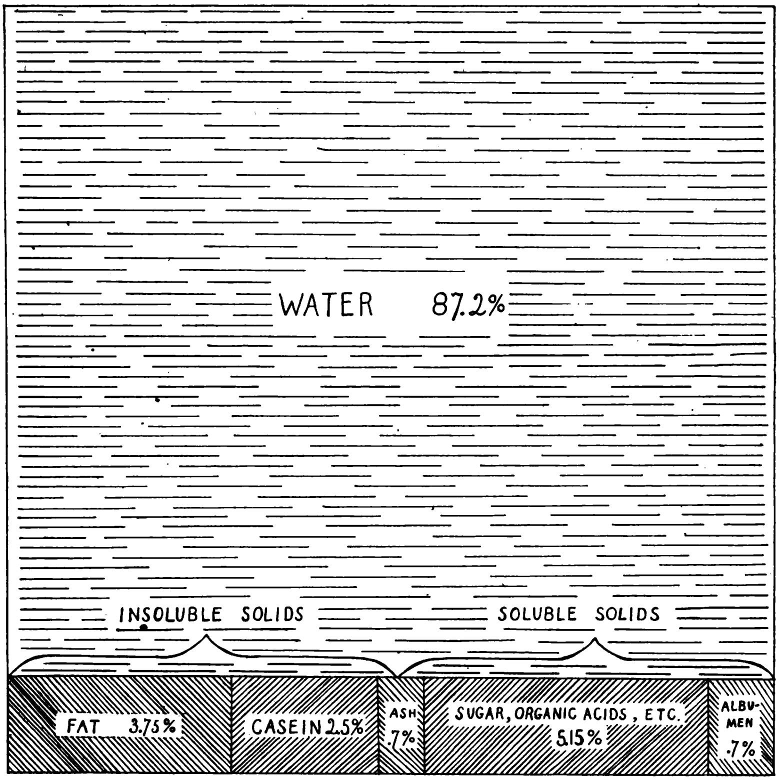


FIG. 1

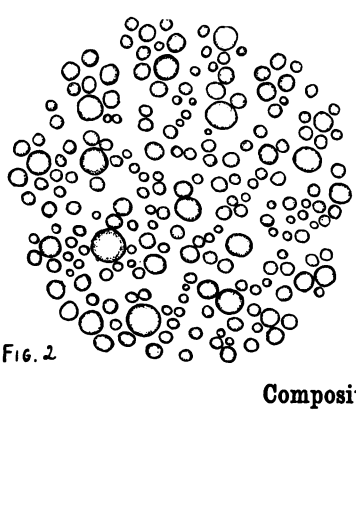


FIG. 2

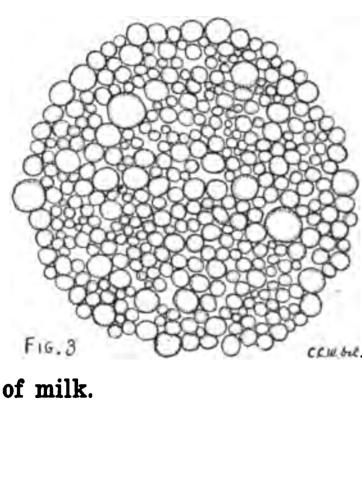
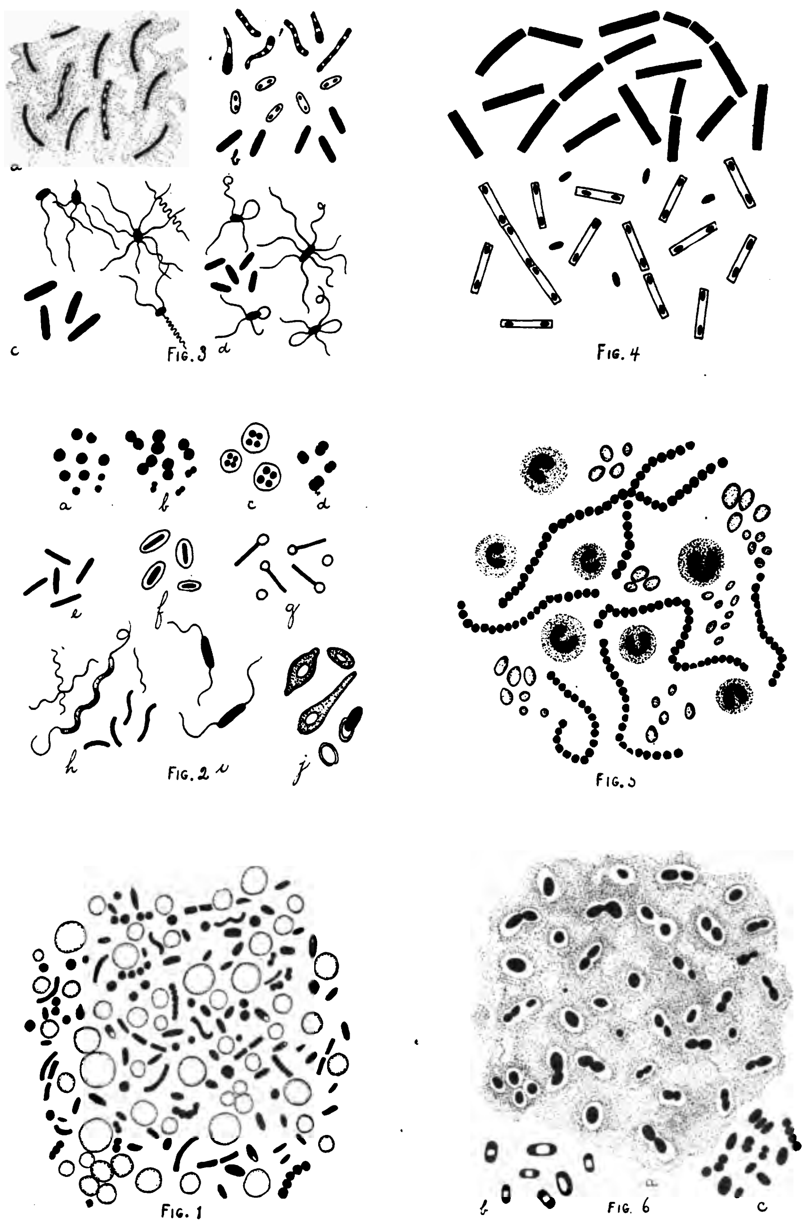


FIG. 3

C.C.W. del.

Composition of milk.

Plate III



Various forms of Bacteria.